Original Article

Effect Of Seed Priming On Germination And Seedling Growth Of Rice (Oryza Sativa) Under Osmotic Stress

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Abstract

One of the prevalent stresses that affects plant growth and development, including crop reductions that are significant all over the world, is drought. One of the most important and complicated physiological processes in a plant's life cycle is seed germination, which is sometimes hampered by environmental and biological stress and results in inconsistent germination. Priming is a classic technique typically employed for synchronized seedling growth and stable crop stand, but priming has emerged as a powerful tool for sustainable agriculture in recent years. In this experiment, the efficacy of seed priming was tested. Seven rice (Oryza sativa L.) varieties were tested for osmotic tolerance and seed priming was done to analyze the increased resilience of primed seeds for osmotic stress. Varying polyethylene glycol (PEG) concentrations (0%, 10%, and 20%) were used to induce osmotic stress in plants. The maximum germination percentage after stress imposition were reported in ADT-39 and ADT-45 compared to the other varieties, and a dramatic decline in germination percentage and increased proline content was seen in the BPT-5204 variety in both primed and non-primed seeds. In all the varieties, osmotic stress induced a decline in seedling growth as measured by shoot and root length, fresh weight, and dry weight. ADT-39 was the most resilient variety exhibiting maximum tolerance to osmotic stress. Along with the increase in PEG concentration, the total amounts of chlorophyll and proline decreased. At each concentration of PEG, the ADT-39 had retained the maximum chlorophyll content. According to the finding of the study ADT-39 is identified as a drought tolerant cultivar exhibiting maximum tolerance at germination stage. Priming technique can be combined with directed seeded sowing practice especially for drought prone areas.

Introduction:

World's agriculture is being threatened by climate change, the fast urbanization-related shrinking of arable land, the unchecked use of chemical fertilisers and pesticides, biotic and abiotic stress. Reduction in yield tempts the farmers to use excessive fertilizers and pesticides and this ultimately poses threat to mankind, declines water quality, eutrophication and biomagnification of the residual lot in food web. The United Nations and Food and Agricultural Policy Research Institute (FAPRI) predict that by 2035, there will be a 555 million tonne worldwide demand for milled rice (Paul et al., 2022). Only by coordinating environmental sustainability, food security, and agricultural output can this paradoxical situation be resolved (Pandey and Diwan 2020, Paul et al., 2022). After wheat, rice is the second-most devoured food grain on the planet. 80% of the energy requirements of more than two billion people in Asia are filled by rice, and it constitutes of 80% carbohydrates, 7–8% protein, 3% fiber and 3% fat (Juliano 1985). In most of the cultivated regions of the world, drought is one of the principal factors decreasing crop yields. Drought condition can arise under several conditions, such as atmospheric (below average rainfall), agricultural (below average water supply), soil-related (lack of surface or groundwater), and physiological (transpiration is greater compared to water uptake) (Wilhite and Glantz et al., 1985). This is one of the most significant abiotic stresses that reduces plant growth and development. There are several techniques to mitigate the negative impacts of drought. Seed germination is one of the crucial event in crop establishment that is prone to various factors like soil pH, temperature, moisture content etc. One effective strategy to deal with drought stresses is seed priming. To improve germination, seedling vigor, and stress tolerance, seeds are partially hydrated as part of the priming process prior to planting. In addition to reducing the time needed for seedling emergence and accelerating germination, priming also helps in improving crop quality and ensures uniform germination. During the priming process, seeds are exposed to outside elements including chemicals, heat, and moisture, which alters their physiological and biochemical condition. The seeds can now withstand environmental challenges like drought, salt, and temperature variations better after priming. Several agricultural crops have benefited from the use of seed priming methods including hydropriming and non-priming, which has been shown to increase drought tolerance, increase the emergence of roots and shoots, and produce healthier plants. (Iqbal and Ashraf, 2007). Rice seed hydropriming with potassium hydro phosphate (KH_2PO_4) can improve germination, seedling emergence, and plant growth.

As reported by Cakmak (2005), crop plants may need to have their K-nutritional balance improved to survive under environmental stress circumstances such as an intense drought. Rice has also been effectively primed using hydropriming and osmopriming techniques (Harris et al., 2001). Additionally, hydropriming stimulated the development of seedlings under osmotic stress compared to non-priming (Kaur et al., 2002; Kaya et al., 2006; Casenave and Toselli, 2007). Polyethylene glycol (PEG) may resemble drought stress during the early stages of growth. In numerous investigations, artificial abiotic stress has been induced using PEG, a high molecular weight osmotic material. Due to its high molecular weight and inability to penetrate into cells, PEG was employed in various experiments.

PEG 6000 was used in the current investigation as it can regulate water potential in germination studies and cause osmotic stress without producing immediate physiological harm (Almaghrabi et al., 2012). The speed and regularity of seedling development and growth are enhanced by priming, especially in stressful situations (Parera and Cantliffe, 1991). By adjusting the osmotic potential of nutrient solution culture, polyethylene glycol (PEG) can be utilized to produce a plant shortage of water in an effectively regulated way (Money, 1989; Zhu et al., 1997). PEG molecules can generate equitable water stress instead of producing immediate physiological harm because they are neutral, non-ionized, and almost impermeable to the membranes of cells (Lu and Neumann, 1998; Kulkarni and Deshpande, 2007). The objective of the study was to determine the impact of osmotic stress on crop growth and crop development, with a focus on seed priming techniques in seven varieties.

Materials and Method:

Plant materials:

Seven cultivated varieties (Bhavani, Thanuska, White Ponni, Aman, BPT-5204, ADT-39, and ADT-45) of rice (Oryza sativa) were procured from Tamil Nadu, India. These varieties are popularly grown in southern part of India (Table 1). Seeds were oven dried at 48°C for 5 days to ensure uniform germination and minimize seed dormancy. In this study, basically priming of seeds were done and germination %, chlorophyll and proline content of seven varieties were tested between primed and non-primed (control) seedlings.

Variety Name	Origin	Duration (Days)	Grain type
Bhavani	Tamil Nadu, India	130-135	Long slender
Thanushka	Tamil Nadu, India	120	Medium slender
White Ponni	Tamil Nadu, India	135-140	Medium slender
Aman	Tamil Nadu, India	120-150	Slender elongated
BPT-5204	Tamil Nadu, India	150	Medium slender

ADT-39	Tamil Nadu, India	120-125	Medium slender
ADT-45	Tamil Nadu, India	110	Medium slender

Table 1: Cultivars details: origin, days to maturity and grain types

General experimental details:

Several priming solutions were used to prime the seeds and examined under controlled conditions to find out how priming affects germination and the emergence of different rice cultivars. However contrasting results were obtained with optimized concentration of KH_2PO_4, Polyethylene glycol-6000 and $Cacl_2$ and hence these are used for priming in the current investigation. So, in this study, we compare the efficacy of primed and non-primed seeds with three replications each. One set of seeds were sown in replicates in petriplates to check the germination % and one set of seeds were grown in hydroponic condition for other biochemical estimations. Seven cultivars' seeds were imposed to stress with PEG 6000 at three different concentrations (0%, 10%, and 20%). In control condition only hydroponic solution was used. For 10% and 20% concentrations, 10g and 20g of PEG 6000 was dissolved with 100 ml of distilled water.

Seed Priming Germination Treatment:

The experiment was conducted in Plant Growth Chamber. 10% and 5% of potassium dihydrogen phosphate (KH_2PO_4) and polyethylene glycol-6000 (PEG-6000) were prepared and used for seed priming. About 50 seeds were placed in 50 ml of water with 10% KH_2PO_4, 5% PEG-6000 and 100 mM $Cacl_2$. The seeds were primed for 24 hours at 36 °C in the dark with constant gentle agitation. The ratio of seed weight to solution volume (w/v) was 1:5. The priming solution was changed every 12 h. Autoclaved distilled water was used for priming. After 24 hours, the seeds were removed from the dark shade, the priming solution was discarded, and seeds were placed into petri dishes containing blotting paper to absorb moisture and the blotting paper was used to remove the absorbed moisture from the seeds and seeds were kept for drying at least for 48 hours, till the moisture content was less than 10%. Both the primed seeds and non-primed seeds were treated with 2% Bavistin for 20 mins. Then the seeds were thoroughly washed with dd H_20 to remove any traces of surfactant fungicide. Then the seeds were sown in petriplates and one set was maintained as control and other two sets were exposed to 10% and 20% PEG-6000 (treatments). Experiment was conducted in replicates and petriplates were placed in an incubator for germination at 36 °C. 12-hour light and 12-hour dark was maintained. Humidity was maintained at 60%.

Root-Shoot length:

Seven varieties were grown in trays with hydroponic solution. One set was maintained as control and other two sets were exposed to varying degree of PEG-6000. One set was exposed to 10% PEG and other to 20% PEG. When the visual symptoms of damage were evident, observations were recorded. On the 14th day germination%, root and shoot lengths (cm) were measured. Five plants were randomly selected from control, 10%, and 20% PEG-6000 concentrations. After excising the plants were thoroughly cleaned with distilled water. After drying with blotting paper, the root and shoot parts were separated from each other to measure the root and shoot length (cm) using the graduated scale. Then the root and shoot lengths were placed in a hot air oven at 70 °C to dry, and an electronic balance was used to measure the dry weight.

Seed germination test data:

Germination of seeds was recorded on daily basis according to AOSA (1990) till it became constant. Seed was considered to be germinated when radicle length exceeded 2 mm. The following information was noted or extrapolated from the germination test (Koirala et al., 2019).

● percentage of germination

● 14 days after sowing, the root length (DAS)

● 14 days after sowing, the shoot length (DAS)

The following formula was employed to determine the germination percentage (GP) (Koirala et al., 2019).

Number of seeds germinate.

GP = -- x 100%

Total number of seeds set for test.

Estimation of Chlorophyll content:

Seven different rice cultivars (Bhavani, Thanuska, White Ponni, Aman, BPT-5204, ADT-39, and ADT-45) were employed for the estimation of chlorophyll. The fresh second leaf of the varieties were used in chlorophyll estimation. The absorbance was taken using a UV spectrophotometer. 25 g of fresh plant leaves were accurately weighed, and 10 ml of pre-chilled 80% acetone was used as the solvent for the extraction (Sarkar et al., 1993). After 48 hours, the analysis of the chlorophyll content was done and the absorbance was recorded at 663.6 nm and 646.6 nm, respectively, and the amount of chlorophyll a and b was determined by the following equations (Sarkar et al., 1993):

Chl a = 12.25 (A 663.6) - 2.25 (A 646.6)

Chl b= 20.31 (A 646.6) - 4.91 (A 663.6)

Total Chl (a+b) (mg/ml) = 17.74 (A646.6) + 7.31 (A663.6)

Estimation of Proline Content:

Free proline content in rice seedlings was determined through Bates et al. (1973) method. Briefly, harvested samples were homogenized in 5 ml of 3% sulfo-salicylic acid and centrifuged at 6,000 rpm for 10 min. Supernatant (2 ml) was heated with 2 ml of ninhydrin and 2 ml glacial acetic acid at 100◦C for 1 h. The reaction mixture was further extracted with 4 ml of toluene by vigorously vortexing for 30 s. The absorption of chromophore was determined at 520 nm (Tecan-infinite M200, Switzerland). The concentration of proline in the samples was estimated by referring to a standard curve of L-proline

Result:

Germination Percentage of Hydropriming and Non-Priming Treatment:

The germination dynamics of primed and non-primed seeds exposed to osmotic stress are shown in figure 1. Priming treatment had a significant impact on germination percentage. In Figure 1, it can be observed that germination percentages of the Bhavani, Thanushka, White Ponni, Aman, BPT-5204, ADT-39, and ADT-45 cultivars were affected by osmotic stress induced by PEG-6000. In control condition (i.e., no stress) highest germination percentages was observed in almost all varieties, while in 20% PEG-6000 lowest germination percentage was observed after 7 days of stress imposition. Germination % was recorded at each day. In 20% PEG concentration, there was minor change in the percentage of seeds that germinated under osmotic stress compared to the control. Among the varieties ADT-39 and ADT-45 are the most tolerant, Bhavani is the medium tolerant and BPT-5204 is the most susceptible under osmotic stress in both non-primed and primed condition. In non-primed seeds, the % reduction in ADT 39 was 6.67% between C and 10%. similarly 6.67% reduction was observed between C and 20%. Additionally, in primed seeds, ADT-39 had no reduction between control and 10% while it was 6.67% reduction between control and 20%. In non-primed seeds, ADT-45 had 6% reduction between control and 10% while it was 13.34% reduction between control and 20%. Furthermore, in primed seeds, ADT-45 had 6.67% reduction while it was 13.34% reduction between control and 20%. In non-primed seeds, Bhavani had 15.9% reduction between control and 10% while it was 22.4% between control and 20%. In primed seeds, Bhavani had reduction of 13.34% between control and 10% while it was 20% between control and 20%. In non-primed seeds, BPT-5204 had 25% reduction between control and 10% while it was 41.67% reduction between control and 20% . But in primed seeds, BPT- 5204 had reduction of 23.07% between control and 10% while it was 38.46% reduction between control and 20%. This result showed that, ADT-39 and ADT-45 had lowest reduction and hence are identified as tolerant genotypes and BPT5204 had highest reduction under osmotic stress (Figure 1).

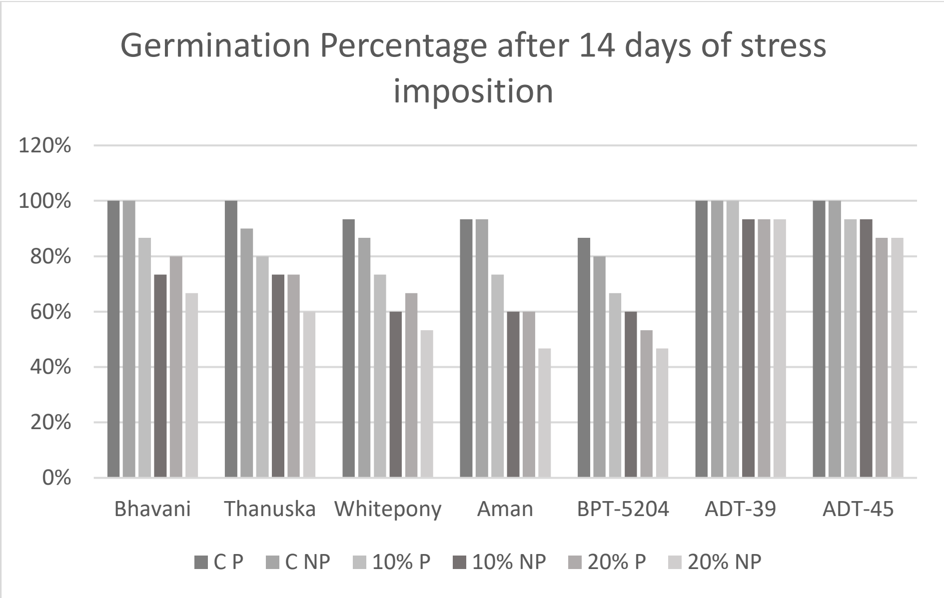

Figure 1. Effects of priming and non-priming on germination percentage (%) of rice seeds under osmotic stresses of PEG. P: priming; NP: Non-priming; Control: only distill water; 10%PEG: distill water+10%peg; 20%PEG: distill water+20%peg.

Root length and shoot length of seedlings after 14 days of treatment:

Priming also had a significant effect on seedling vigor, seeding emergence, panicle length and radicle length. Root and shoot length reduction was less in primed seedlings compared to non-primed seedlings. At 10% and 20% concentrations substantial differences in root and shoot length compared to the control concentration. Among the varieties ADT-39 and ADT-45 are the most tolerant genotypes, Bhavani is the medium tolerant and BPT-5204 is the most susceptible under osmotic stress. In non-primed seeds, the % reduction of the root length in ADT 39 was 4.4% between C and 10%, while it was 9.3% reduction between control and 20%. Additionally, the % reduction of the shoot length in ADT-39 was 2.72% between control and 10% while it was 8.1% reduction between control and 20%. In the primed seeds, the root length of ADT-39 had 3.8% reduction between control and 10% while it was 8.1% between control and 20% (Figure 2 and 3). Additionally, the shoot length of ADT-39 had 2.67% reduction between control and 10% while it was 5.3% between control and 20%. In non-primed seeds, the root length of ADT-45 had 5.5% reduction between control and 10% while it was 11.1% between control and 20%. The shoot length of ADT-45 had 4.8% reduction between control and 10% while it was 9.6% between control and 20%. In primed seeds, the root length of ADT-45 had 4.2% reduction between control and 10% while it was 9.6% between control and 20%. The shoot length of ADT-45 had 4.76% between control and 10% while it was 7.61% between control and 20%. In non-primed seeds, the root length of Bhavani had 5.6% reduction between control and 10% while it was 14.3% between control and 20%. The shoot length of Bhavani had 10.11% reduction between control and 10% while it was 13.4% between control and 20%. In the primed seeds, the root length of Bhavani had 4.34% reduction between control and 10% while it was 13% between control and 20%. When primed ADT 39 and ADT 45 are better performers.

In non-primed seeds, the root length of BPT-5204 had 14.6% reduction between control and 10% while it was 31.7% between control and 20%. The shoot length of BPT-5204 had 24.52% reduction between control and 10% while it was 39.62% between control and 20%. In the primed seeds, the root length of BPT-5204 had 13.1% reduction between control and 10% while it was 31.3% between control and 20%. The shoot length of BPT-5204 had 21.31% reduction between control and 10% while it was 39.34% between control and 20% (Figure 2 and 3).

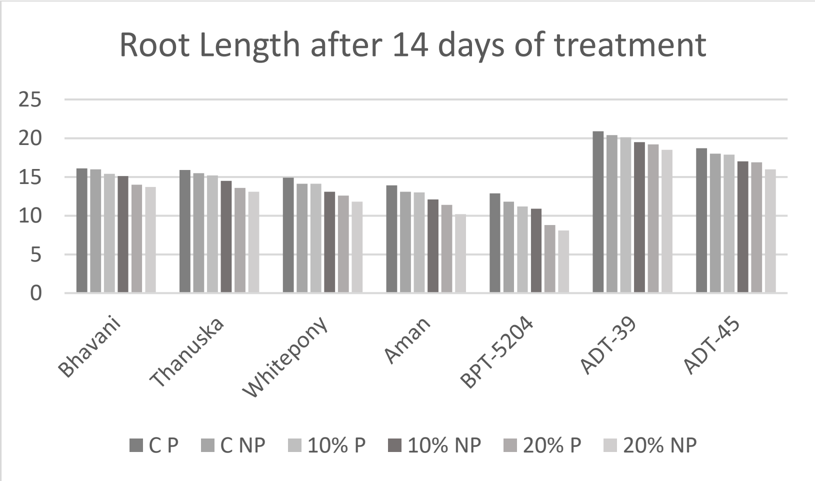

Figure 2. The effects of priming and non-priming on root length (cm) of rice seeds under osmotic stresses of PEG. P: Priming; NP: Non-priming; Control: distill water; 10%PEG: distill water+10%peg; 20%PEG: distill water+20%peg.

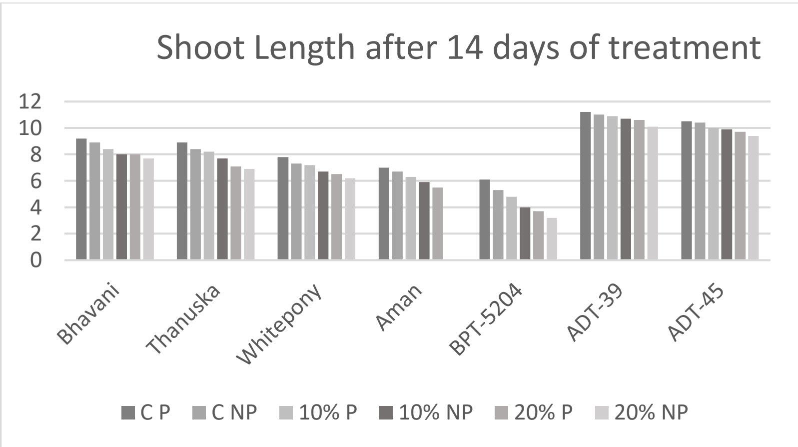

Figure 3. The effects of priming and non-priming on shoot length (cm) of rice seeds under osmotic stresses of PEG. Note: P: Priming; NP: Non-priming; Control: distill water; 10%PEG: distill water+10%peg; 20%PEG: distill water+20%peg.

Dry shoot weight of Seedling after 14 days of stress exposure:

The reduction of fresh and dry weight of shoot was less in primed seedlings in comparison with non-primed seedlings. In 20% PEG concentration, there was significant change in the reduction of seeds under osmotic stress compared to the control. Among the varieties ADT-39 and ADT-45 had the lowest reduction and BPT-5204 had the highest reduction under osmotic stress. The dry weight of ADT-39 had % reduction of 5.17% and 7.14% between control and 10%, respectively and 8.62% and 9.09% reduction between control and 20%, respectively. ADT-45 had reduction of 5.35% and 7.69% between control and 10% while theirs were 10.71% and 14.50% between control and 20%, respectively. BPT-5204 had 7.89% and 9.09% reduction between control and 10%, while theirs were 18% and 21.05% reduction between control and 20%, respectively. This result showed, ADT-39 and ADT-45 is the most tolerant and BPT-5204 is the most susceptible under osmotic stress. (Figure 4).

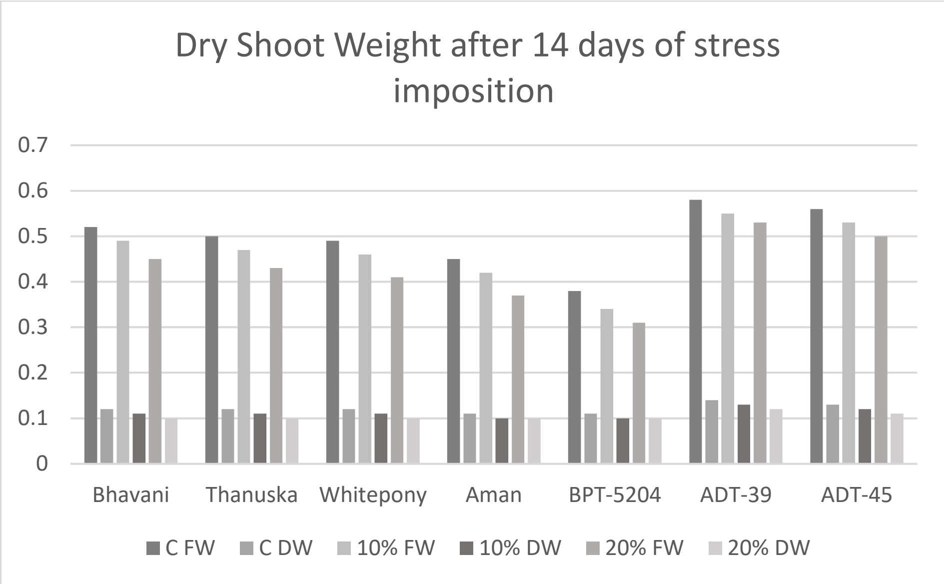

Figure 4. Fresh and dry shoot weight of rice seeds under osmotic stresses of PEG. Note: P: Priming; NP: Non-priming; FW: fresh shoot weight; DW: dry shoot weight; Control: distill water; 10%PEG: distill water+10%peg; 20%PEG: distill water+20%peg.

Chlorophyll content in 7 different rice cultivars under osmotic stress:

Chlorophyll reduction was less in primed seedlings compared to non-primed seedlings. In control concentration reduction was the lowest compared to the 10% and 20% PEG concentrations in both primed and non-primed seeds under osmotic stress. BPT-5204 had the highest reduction and ADT-39 and ADT-45 had the lowest reduction among the varieties. In the non-primed seeds, BPT-5204 had 28.88% reduction between control and 10% while it was 46.6% between control and 20%. In the primed seeds, BPT-5204 had 24.52% reduction between control and 10% while it was 41.5% between control and 20%. In the non-primed seeds, ADT-39 had 7.2% reduction between control and 10% while it was 13.40% reduction between control and 20%. In the primed seeds, ADT-39 had 4.04% reduction between control and 10% while it was 10.10% between control and 20%. In the non-primed seeds, ADT-45 had 9.4% reduction between control and 10% while it was 15.78% between control and 20%. In the primed seeds, ADT-45 had 7.29% reduction between control and 10% while t was 11.45% reduction between control and 20%. The result showed, ADT-39 and ADT-45 is the most tolerant and BPT-5204 is the susceptible under osmotic stress (Figure 5).

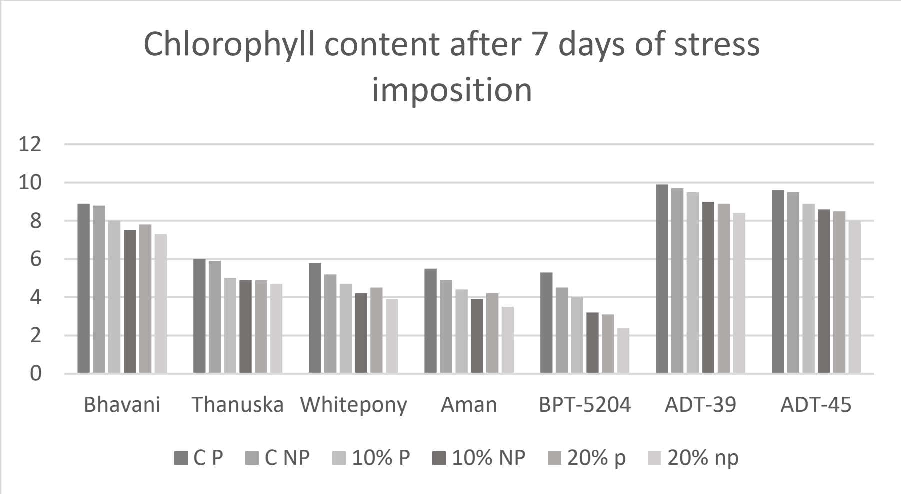

Figure 5. Clorophyll content in rice seeds under osmotic stress. Note: P: Priming; NP: Non-priming; Control: distill water; 10%PEG: distill water+10%peg; 20%PEG: distill water+20%peg.

Proline content in 7 different rice cultivars grown under osmotic stress:

Under conditions of PEG-induced osmotic stress, proline reduction was reduced in primed seedlings compared to non-primed seedlings. Among the varieties ADT-39 and ADT-45 had less reduction and BPT-5204 had the highest reduction in both priming and non-priming treatment. In the non-primed seeds, ADT-39 had 5.58% reduction between control and 10% while it was 9.37% between control and 20%. In the primed seeds, ADT-39 had 4.02% reduction between control and 10% while it was 8.54% between control and 20%. In the non-primed seeds, ADT-45 had 6.48% reduction between control and 10% while it was 10.81% between control and 20. In the primed seeds, ADT-45 had 5.82% reduction between control and 10% while it was 10.58% between control and 20%. In the non-primed seeds, BPT-5204 had 12.97 reduction between control and 10% while it was 23.66% between control and 20%. In primed seeds, BPT-5204 had a reduction of 11.51% between control and 10% while it was 20.14% between control and 20%. The result showed, ADT-39 and ADT-45 is the most tolerant and BPT-5204 is the susceptible tolerant under osmotic stress (Figure 6).

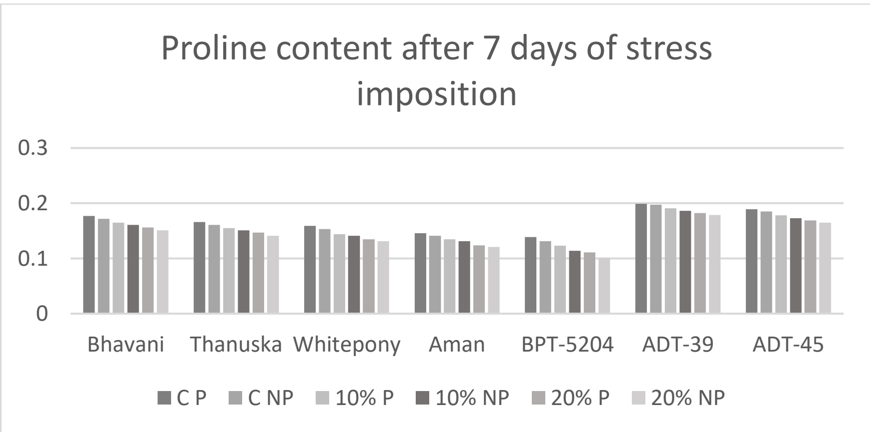

Figure 6. Proline content under osmotic stress. Note: P: Priming; NP: Non-priming; Control: distill water; 10%PEG: distill water+10%peg; 20%PEG: distill water+20%peg.

Discussion:

In several nations, priming seeds before sowing is a common practice for some vegetable and flower crops (Mittal et al., 1995, Bohnert et al., 1999, Liu et al., 2002). The purpose of seed priming is to start the repairing system (Yuan et al., 2010). As a result, it is possible to promote seed germination and tolerance to unfavorable conditions via regulating the seed's rate of water absorption, for membrane, and the metabolic setup for germination. Germination is an important phase in plant growth. In this research, rice seedling germination in hydropriming and non-priming treatments was observed. Several studies have shown that several types of seed priming increase seed germination percentage and seedling emergence (Srivastava et al. 2010). Under osmotic-stress conditions, hydropriming typically has a significant impact on the germination rate and uniformity of the emergence of seedlings (Wahid et al. 2007). Crop field productivity is improved by additional seed priming (Giri and Schillinger 2003). Our research examined hydropriming and non-priming techniques and their effects on germination. Hydropriming seeds with PEG and 5% KH_2PO_4 increased germination percentages in comparison with non-priming seeds. Moreover, the priming technique had a favorable effect on the growth rate. Hydro-primed seedlings were shown to have longer shoot and root lengths than non-primed seedlings under osmotic stress. Although it was determined that hydropriming was more effective for growth under osmotic stress.

In almost all the varieties the effect of 20% PEG concentration and 10% concentration had less impact on seedling growth in priming condition. In this study, after 14 days of germination, the length of the root and shoot, the fresh and dry weight of the shoot were less impacted by the osmotic stress in primed seedlings. According to the research, the PEG solution had a significant impact on the root and shoot lengths. In addition to the 20% concentration of PEG solution, root growth was reduced. While under osmotic stress, similar results were confirmed by Y. Kato et al., (2010). Several studies that have been reported suggest that rice varieties under drought stress exhibit variations in root length (Saha et al., 2019; Abd Allah et al. 2010). In this study, the root and shoot lengths at 10% and 20% concentrations were shorter compared to the control concentration in both primed and non-primed conditions. This indicates that a 20% PEG concentration was able to retard root elongation as a part of adaptation to osmotic stress compared to a 10% concentration. A plant would find it easier to access water sources if its roots were longer (Y. Kim et al., 2020). According to this study, the fresh weight of the shoot at 0%, 10%, and 20% concentrations was higher than the dry weight of the shoot lengths. High chlorophyll content in the tolerant genotypes confirms the intact photosynthetic integrity of Photosystem II contributes to resilience against osmotic stress.

In India, direct seeding is currently employed to produce rice while conserving labor. Uneven seedling growth and low seed germination rates are significant issues in this technique. To ensure the density of seedlings in the field, farmers frequently increase the amount of seed. As a result, the price of seeds rises. Direct rice seeding or paddy rice seedling development systems can both incorporate the seed priming stage. The right priming treatment can promote seed germination, enhance seedling quality, and increase seedlings' ability to withstand drought. These benefits directly lead to an increase in germination, seedling, and vigour seedling rates, which may be a way to reduce seed production. The best priming inducer concentration appears to be related to specific rice varieties. Rice varieties are shown to be related with the best priming inducer concentration. Hence, it should be decided before being put into practice. The period of seed priming is another element to take into account next to the beds. The impact of various priming durations on seed germination and physiological traits of seedlings has to be further investigated because we haven't included more priming durations in our experimental design. Due of the complicated environment of a rice field, we are unsure if the performance of rice seedlings after priming treatment will be as good as the results in our study. Therefore, before using seed priming technology in the production of rice, we advise optimizing it.

Conclusion:

It was found that the exogenous application of different substances, particularly water, had significant effects on the seedlings' growth rates during drought. Although seed pretreatments were found to be quite beneficial for the seedling's establishment, survival, and growth, they were not particularly useful for seed germination. For all the treatments, the priming solution was determined to be the best formulated solution for plants to survive drought stress. Estimates of chlorophyll and proline served as indicators of drought survival. Our data thus provide a major and beneficial contribution to the current body of knowledge regarding crop improvement by elucidating the principles underpinning simple economic ways of seed priming in crucial crop plants like rice. Priming treatment improved metabolism, germination, and seedling quality under drought stress or artificially induced osmotic stress in all cultivars. However, such effect had limited capability, and severe drought stress also caused damages of rice seedlings even though the seeds were treated with optimal treatment of PEG priming. Rice cultivars had significant impact on priming effect, and indica rice showed better performance.

Declarations:

Conflict of interest

The authors declare that there is no conflict of interest

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Authors Contributions

Nahid Anjum, Nishat Anjum, Sharif Mainul Islam - performed the research work, Draft of the manuscript preparation.

Anuprita Ray- Editing of the manuscript.

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